Mycorrhizal mycelia and nutrient cycling in plant communities

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SUMMARY

1 This paper describes some of the structural and functional attributes of the external mycelium of mycorrhizal roots.

2 Root chambers have been employed to investigate the development of the mycelia of both ecto- and vesicular-arbuscular mycorrhizal systems and the observations have been supplemented with simulated and actual field experiments.

3 It is demonstrated that hyphae of the external mycelium of both ecto- and VA mycorrhizal fungi can initiate mycorrhizal infection in intra- and interspecific combinations of host plants.

4 This pattern of infection development leads to the establishment of a persistent network of hyphal interconnections between plants.

5 Using $^{14}CO_2$ it is shown that carbon moves freely between plants connected by the mycorrhizal mycelium and that the movement occurs along concentration gradients which can be induced by shading.

6 It is concluded that these attributes of the mycorrhizal system will improve the vigour of the individual receiver plant, optimize the efficiency of resource distribution within the plant community and enhance the conservation of nutrients at the ecosystem level by restricting losses caused by immobilization in the general soil microflora or by leaching.

INTRODUCTION

Colonization of the terrestrial environment by early land plants would be expected to lead to an increased supply of energy-rich substrates to heterotrophic micro-organisms. Loss of materials from roots would constitute one of the most important supply processes and this would in turn lead both to increased microbial activity and to an intensification of interspecific competition in the zone which we now recognize as the rhizosphere. In these circumstances micro-organisms with the ability to enter into a closer relationship with the autotroph, either as internal or surface occupants of the root, would be at a great advantage since they would obtain direct access to assimilate supplies. Evidence that such close associations were formed by the earliest land plants comes from studies of the roots of fossil pteridophytes of the Devonian period in which vegetative structures similar to those seen in present day VA mycorrhizas can be seen (Nicolson 1975). Since the roots of these early land plants were very poorly developed, exploitation of the soil

environment would be inefficient and mycelial systems growing from their carbon sources into soil would provide significant improvement of absorptive efficiency, so providing a truly mutualistic relationship. This type of root–fungus association was clearly favoured by selection since most individuals in present day natural plant communities are mycorrhizal. Thus, in the soil environment one of the most fundamental interactions is that between roots as primary sources of energy and their mycorrhizal fungi. As a result of their access to this energy source the mycorrhizal fungi possess major competitive advantages over other members of the soil microbial population, and the mycelial networks which they produce must be seen not only in terms of their roles as nutrient absorbing systems but as being themselves a major nutrient resource for the whole soil biota.

Distinct types of mycorrhizal structure are associated with particular soil and vegetation systems (Read 1984). The most widespread type is the vesicular-arbuscular (VA) mycorrhiza, seen in the early land plants, and today occurring in the majority of herbaceous and graminaceous species of temperate (Read, Kouchecki & Hodgson 1976) and semi-arid (Trappe 1981) grassland ecosystems as well as in many tree species of tropical and subtropical forests (St John 1980; Janos 1980). In VA mycorrhizas, in addition to an internal mycelial phase with characteristic storage structures known as vesicles and much branched intracellular hyphal systems called arbuscules, there is an important external phase made up of branched single hyphae which ramify through the soil, forming anastomosing networks. The fungi forming this type of mycorrhiza are zygomycetous members of genera such as *Glomus*, *Gigaspora* and *Acaulospora*. In contrast, the dominant trees of boreal and temperate forest zones have distinct ecto- or sheathing mycorrhizas formed largely by Basidiomycetes, the vegetative mycelium of which is predominantly external with a compact sheath around lateral roots, no intracellular penetration, and frequently with an extensive mycelial phase made up of strands which spread for considerable distances through soil.

The fungi associated with both these types of mycorrhiza show little evidence of host-specificity. Thus, a typical VA fungus such as *Glomus fasciculatum* will form mycorrhizal associations with a range of grass and herb species growing together in a grassland sward. Similarly, in a forest ecosystem an ectomycorrhizal fungus such as *Amanita muscaria* will form mycorrhizas with a number of coniferous and deciduous host trees. The fact that specificity is generally lacking has profound implications both from the point of view of the infection process and in relation to nutrient cycling. The chance that uninfected roots will make contact with compatible mycelium are high under these circumstances. Also, if mycorrhizal infection arises from such contacts the consequence is that roots become physically incorporated into an established mycelial network. If, in turn, this network provides functional pathways for transfer of nutrients between host plants it will be of fundamental importance in relation to nutrient cycling processes at the ecosystem level.

Unfortunately, because of the fragile nature of the mycelial systems it is difficult to investigate its biology in a non-destructive manner in the field. Using observation

chambers it is, however, possible to analyse the development and function of the mycorrhizal network with appropriate combinations of host plants, fungi and soils in the laboratory. The results of such experimental analyses are reported here.

MYCELIA OF ECTOMYCORRHIZAL PLANTS

Experimental methods

Mycorrhizas were first synthesized under sterile conditions using aseptically germinated seedlings of a range of host tree species and pure cultures of mycorrhizal fungi isolated from fruit bodies. The mycorrhizal plants were then transferred to observation chambers made of transparent perspex and containing unsterile peat or forest soil. In such chambers the progress of development of the mycorrhizal mycelium can be followed as it colonizes the fresh soil, and its capacity to initiate infection in newly developed roots of the original host or on other plants introduced into the chamber in intra- and interspecific combination can be determined. Details of the chamber systems are provided in Brownlee *et al.* (1983).

Some of the physiological functions of the external mycelium have been investigated using $^{14}CO_2$ as tracer. In these experiments shoots of individual plants selected to act as isotope 'donors' were sealed into transparent chambers and appropriate quantities, usually in the range 20–100 μCi, of $^{14}CO_2$ were released into the chambers. After a period of 24–72 hours, which is normally sufficient to ensure that complete utilization of $^{14}CO_2$ has occurred, the donor shoot system was removed and the chamber carefully opened. Where distribution of isotope was to be determined by autoradiography, the chambers were rapidly dried at 60°C and incubated in contact with X-ray film. Where quantitative distribution of isotope was to be carried out the shoots or roots were ground in liquid nitrogen and aliquots of the extract were digested in NCS tissue solubilizer prior to being transferred to liquid scintillant for counting on a Packard Liquid Scintillation counter.

Analysis of ectomycorrhizal systems in observation chambers

In plants like pine and birch mycorrhizal roots act as point sources of inoculum from which mycelia spread outwards in fan-shaped formation to colonize surrounding soil (Fig. 1a, b). While in young fans most of the mycelium is in the form of individual hyphae (Fig. 2a), aggregation of parallel running hyphae takes place as they extend, so that fans which are more than 3–4 weeks old contain many macroscopical strands (Fig. 3b). A feature of this type of mycelial development is the very high density of fungal hyphae in the soil, especially in the most diffusely branched region of the fan in the region of its leading edge. This pattern of growth clearly provides complete exploitation of the soil through which it passes and forms an extremely effective means of scavenging for nutrients, water, or uninfected roots. When the leading edge of an extending fan makes contact with an uninfected root, whether in the same plant (Fig. 3a) on another plant of the same species (Fig.

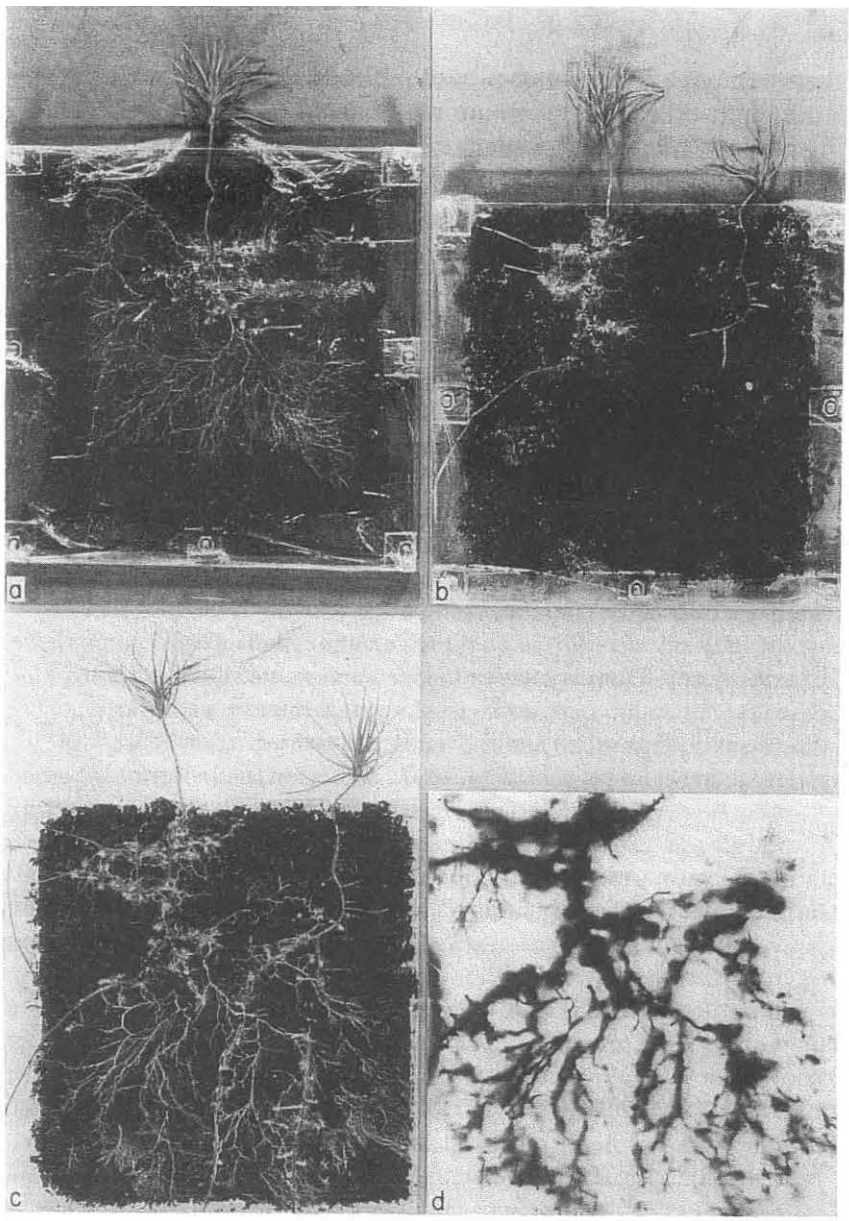

FIG. 1. Showing the development of ectomycorrhizal mycelium in soil, the formation of interplant connections and the direct transfer of isotope between plants. (a) Seedling of *Pinus sylvestris* 4 weeks after transfer to unsterile peat in a root chamber. Mycorrhizas had first been synthesized under aseptic conditions using a pure culture of the fungus *Suillus bovinus*. The fan-shaped arrangement of the mycorrhizal mycelium provides very effective exploitation of the peat. (b) Mycorrhizal seedling of *P. sylvestris* (left) 10 days after transfer to root chamber and non-mycorrhizal seedling of the same species (right). Initial stages of colonization of peat can be seen in the mycorrhizal plant. (c) The same chamber 5 weeks later. The fans from the mycorrhizal plant have extended to exploit the whole chamber. In so doing they have made contact with the non-mycorrhizal plant and established infection over much of its root system. (d) Autoradiograph of the same association prepared 24 hours after feeding $^{14}CO_2$ to the more mature plant. Label has been transferred to the entire mycelial network and has accumulated particularly in the young mycorrhizal roots of the 'receiver' plant.

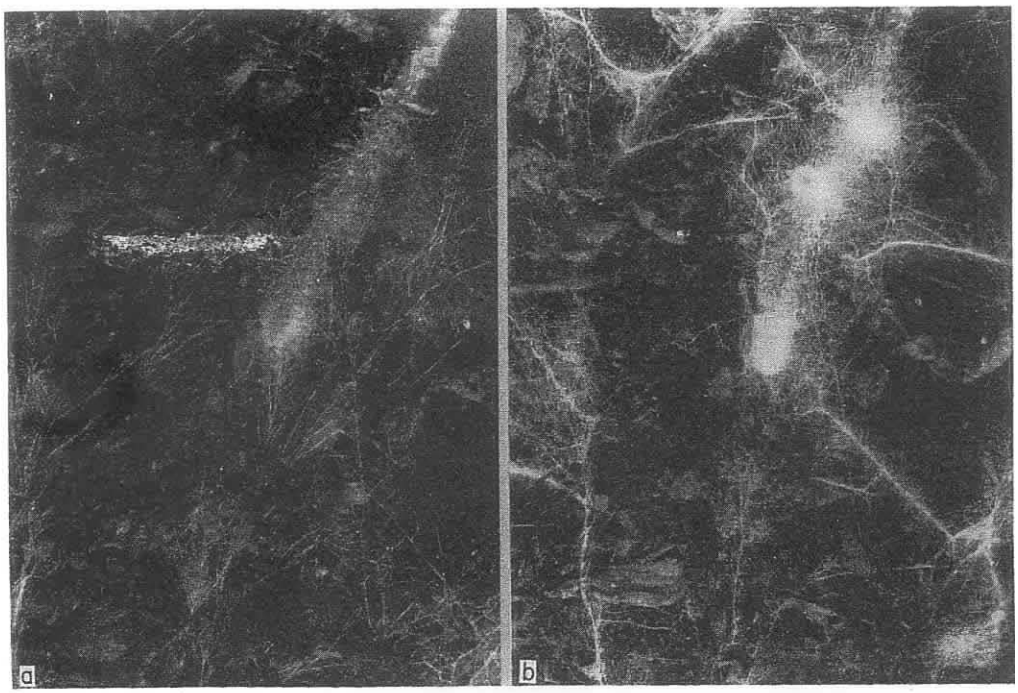

FIG. 2. Magnified view of zone of contact between the leading edge of a fan of mycorrhizal hyphae and an uninfected root showing stages of mycorrhiza formation. (a) Many individual hyphae contact the root surface. Some evidence of proliferation of hyphae is evident at the root apex. Root hairs are visible behind the apex. (b) The same root 2 weeks later showing complete sheath formation at the apex and on two newly-formed lateral roots. Agregation of mycelium around some of the individual hyphae shown in (a) has led to formation of distinct strands. These form the major interconnections through the fan to the original 'donor' root (× 20).

1b, c), or on a different but compatible host species, mycorrhizal infection is rapidly established (Fig. 2a, b). Close examination of this process reveals that following the establishment of a new infection enhancement of strand development occurs in those parts of the fan which first contacted the root (Fig. 2b). As these strands develop, the remaining parts of the fan break down so that within a few weeks of initiation they are difficult to recognize in their original form. The result of this pattern of root colonization and strand growth is that major strands form interconnecting bridges between roots which eventually become the only structures visible in the soil (Fig. 3b). These structures clearly have the potential to act as pipelines for the distribution of nutrients between plants.

Autoradiographs of such systems prepared after feeding shoots of 'donor' plants with $^{14}CO_2$ confirm that the strands act as channels for the direct transfer of carbon from plant to plant (Fig. 1d). In addition, perhaps more importantly from the point of view of the soil biota as a whole, the assimilates are distributed throughout the entire network of advancing hyphae, label being detected in the mycelia within a few hours of feeding to the shoot. The macroscopic parts of

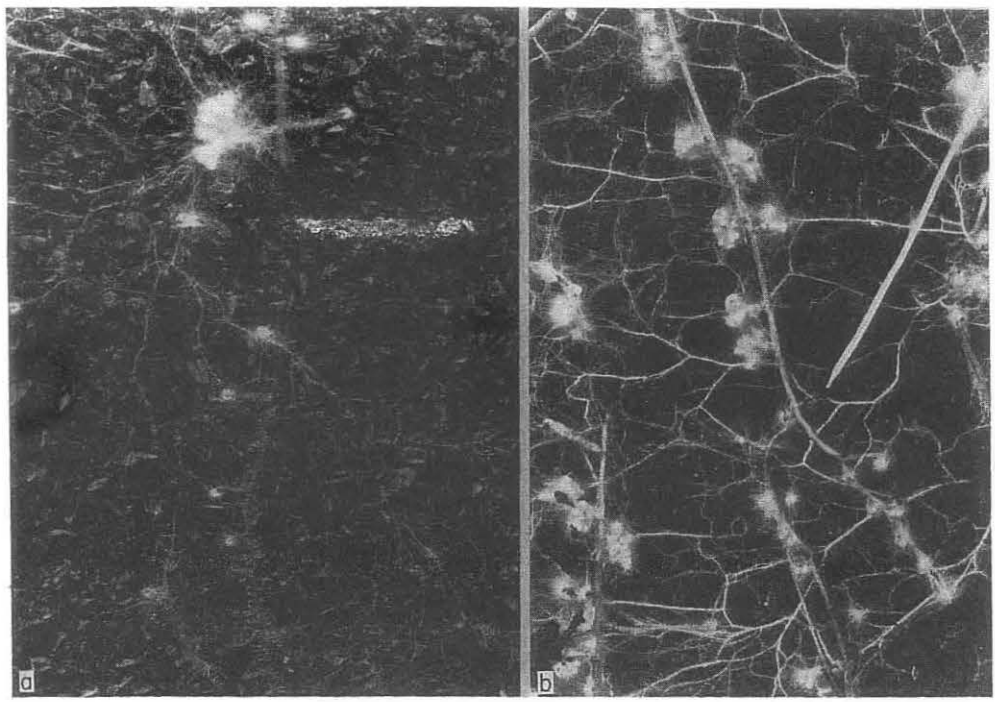

FIG. 3. Levels of strand formation in soil. (a) Strands developing from an established mycorrhizal root cluster act as sources of inoculum on emerging laterals further along the same root system. All mycorrhizal laterals thus become interconnected by strands (× 5). (b) Mature region of root chamber showing complete exploitation of the root environment by anastomosing network of strands (× 10).

mycelia have been harvested after such a feeding experiment and the major labelled product has been shown to be trehalose (D.J. Read, unpubl.). Current assimilates are thus rapidly translocated over large distances from roots into soils through the mycelial network. Where interconnections have formed between roots it is clear that the newly-formed mycorrhizas of the 'receiver' plant are major sinks for the carbon (Fig. 1d). Quantitative determination of the radioactivity (Table 1)

TABLE 1. The distribution of radioactivity (d.p.m. per mg dry wt) in roots and shoots of mycorrhizal (M) and non-mycorrhizal (NM) *Pinus contorta* plants grown in association with 'donor plants' of the same species

	Shoot	Root	Activity in whole receiver plant as % of that in donor
NM	98·2	304·3	0·028
M (*Suillus bovinus*)	304·9 ($P < 0·05$)	3640·7 ($P < 0·01$)	0·234
M (*Suillus granulatus*)	145·4 (NS)	1569·4 ($P < 0·01$)	0·216

Donor plants were fed with 50 μCi of $NaH^{14}CO_3$ for 72 hours.

Significance levels refer to differences between NM and M treatments and are based on analysis of variance of ln-transformed d.p.m. data.

TABLE 2. Host and fungus combinations in which interplant mycorrhizal connections have been synthesized and $^{14}CO_2$ transfer demonstrated (+). Cases where mycorrhizas have not formed in the 'receiver' are indicated with zero (0). Dash (−) indicates synthesis not attempted

Donor species	Receiver species	Fungal species					
		Amanita muscaria	*Rhizopogon roseolus*	*Paxillus involutus*	*Suillus granulatus*	*Suillus bovinus*	*Suillus luteus*
Pinus sylvestris	*Pinus sylvestris*	+	+	+	+	+	+
	P. contorta	+	+	+	+	+	+
	Picea abies	+	+	+	0	0	0
	P. sitchensis	+	0	+	0	−	−
	Betula pubescens	+	+	+	0	0	0
Pinus contorta	*Pinus contorta*	+	+	+	+	+	+
	P. sylvestris	+	+	+	+	+	+
	Picea abies	+	+	+	0	0	0
	P. sitchensis	+	+	+	0	0	0
	Betula pubescens	+	+	+	0	0	0

shows that significantly larger amounts of label occur in both shoots and roots of M than in those of NM plants. In the cases which have so far been examined this pattern of distribution between plants appears to be repeated when the linkages occur at the interspecific level (Table 2).

In addition to the transfer of carbon from autotroph to the advancing hyphal front, it has been shown that water moves in the opposite direction (Duddridge, Malibari & Read 1980), presumably along a gradient of water potential from soil to the transpiring plant. Analysis of the structure of mature strands of *Suillus* species reveals that they are differentiated structures with central 'vessel' hyphae of large diameter which lack cytoplasm and cross walls, surrounded by dense cytoplasmic sheathing hyphae of narrower diameter (Duddridge *et al*. 1980). A similar structure has been observed in strands of *Rhizopogon* (Foster 1981). Clearly the 'vessel' hyphae have a much greater hydraulic conductivity than the sheathing hyphae and it is likely that they function as the major channels of water and solute transport. They are therefore in functional terms analogous to xylem elements. Carbon transport, occurring in the opposite direction, must be restricted to the outer elements of the strand which are therefore analogous in function to phloem. The strands are thus functional extensions of the root system to which they are attached.

Field studies

It is clearly desirable to extend such laboratory investigations to forest situations but the complexity of root distribution and the delicacy of mycorrhizal mycelia make this extremely difficult. However, an investigation of the pattern of carbon transfer between mature trees and naturally regenerating plants has recently been made in an area with 35 year old, 15 m high trees of *Pinus contorta* in which each tree was surrounded by a sward of naturally regenerating plants (D.J. Read, unpubl.). The regenerating plants were of mixed species, most being of *P. contorta* growing under the parent trees, but together with these were young plants of *Pinus sylvestris, Picea sitchensis, Betula pubescens, Chamaecyparis lawsoniana* and *Ilex aquifolium*. The crowns of selected large *P. contorta* trees were enclosed in purpose-built polythene sacks in July, the remaining branches of the tree were removed, and 20 mCi of $^{14}CO_2$ was released into the sack. During the period of feeding, the crowns of some of the naturally regenerating *P. contorta* plants were loosely covered with black polythene sacks to increase shade. The base of the 'donor' tree was checked for signs of radioactivity for a period after feeding using a portable Geiger-Muller radiation monitor. The first indication of the presence of activity at ground level was obtained 8 weeks after release of the isotope.

At this stage selected main roots of the regenerating trees around each 'donor' were excavated to the points at which healthy fine roots could be collected. Approximately 100 ectomycorrhizal roots of shaded and of unshaded *P. contorta* were sampled around each donor. Forty roots of each of the other species in the area were also excised. These included roots of *C. lawsoniana* and *I. aquifolium* both of which are VA mycorrhizal species. All roots were transferred to the

laboratory where levels of radioactivity were determined by the method described earlier.

Not surprisingly variation between individual roots of receiver plants was very large, some showing no activity while others were highly radioactive. This variability probably arises through a combination of factors, not least of which will be the difficulty of sampling roots in such a heterogeneous environment, a random pattern of distribution of mycelial connections between the different trees and variation in the physiological ages of the roots. Because of the variability only the highest levels of activity found on a given individual receiver tree are shown (Fig. 4). Considerable quantities of carbon have been transported to some trees and the fact that highest levels are found in receivers which had been artificially shaded suggests that the process was under physiological control. The absence of activity from all roots of the VA plants *C. lawsoniana* and *I. aquifolium* further indicates that transfer was through ectomycorrhizal interconnections. Experiments of this type provide indications that the processes observed in the laboratory are found also in the field, but they also highlight the difficulties of making meaningful measurements of mycelial transfer processes in the mature forest.

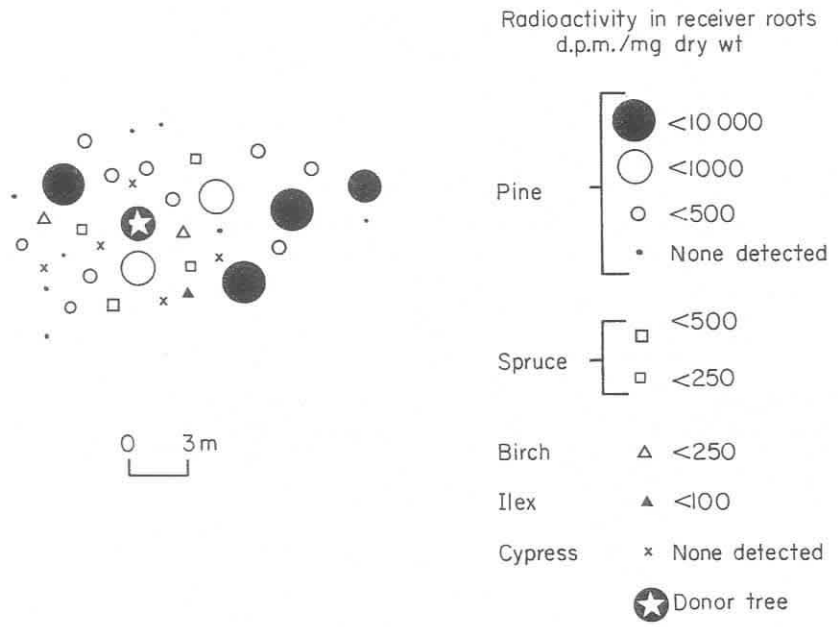

FIG. 4. The distribution of radioactivity in a mixed species group of regenerating 'receiver' plants growing around a mature *Pinus contorta* tree, the crown of which had been exposed to 20 mCi of $^{14}CO_2$ 8 weeks previously. All trees marked were sampled and the distribution of the highest levels of activity is shown.

MYCELIA OF VESICULAR-ARBUSCULAR MYCORRHIZAS

Experimental methods

Since the network of VA mycelium in soil is made up of single hyphae, methods for study of its form and function require microscopic techniques. In our studies, mycorrhizas were first synthesized by inoculating 'donor' seedlings of *Plantago lanceolata* either with surface sterilized spores, or with a mixed inoculum of VA fungi on infected root pieces collected from the field. After infection had occurred the mycorrhizal (M) plants were transferred to shallow transparent dishes containing a 1 cm layer of sterilized or unsterilized dune sand. Young non-mycorrhizal seedlings of the same species or of *Festuca ovina* were then introduced into the dishes. A parallel series of dishes was established in which the 'donor' plants were non-mycorrhizal (NM). After 6–8 weeks of incubation in a growth room the M and NM dishes were divided into two sets, one of which was used for examination of the pattern of development of the mycelium and the other for analysis of viability and function of the network using $^{14}CO_2$ as a tracer.

In order to determine the distribution of mycelium the horizontal walls of the dishes were cut away. Loose superficial layers of sand were then removed by means of a fine jet of water. Below the surface the grains are held by the meshwork of mycelium. Adhering sand grains were removed individually under a dissecting microscope using storksbill forceps. Some breakage of hyphae is inevitable, but since most of the mycelium develops on the transparent base of the dish the network remains intact and its arrangement relative to roots of the host plants and the soil can be analysed and photographed.

Shoots of donor plants of both M and NM treatments were exposed to $^{14}CO_2$ in sealed chambers. Where distribution of radioactivity was to be assessed by autoradiographic means the procedure described above for display of the mycelium was repeated. The freshly excavated system was first photographed and then exposed to autoradiographic stripping film (Kodak AR10). Film was removed and developed after 2 days of incubation and the distribution of radioactivity was analysed with reference to the photographs taken earlier.

Where radioactivity was to be determined quantitatively, pots or seed trays were used instead of transparent dishes. The surface of all pots was covered with paraffin wax before exposure of donor plants to $^{14}CO_2$ in order to eliminate transfer of gaseous $^{14}CO_2$ from the soil to the shoots. In the pot experiments shoots of some M and NM receiver plants were covered by loosely fitting caps which reduced light levels by half or to total darkness. In these experiments the donor plants was *Plantago lanceolata* and receivers were seedlings of *Festuca ovina*.

A simple sward situation was simulated by growing seedlings in a seed tray around a relatively mature plant. Groups of seedlings of *Plantago lanceolata* which were to be used as 'donor' plants were first grown in either the mycorrhizal (M) condition by inoculation with infected root pieces, or in the non-mycorrhizal (NM) condition. After the establishment of mycorrhizal infection in the M plants, both M

and NM *Plantago* were transferred to a central position in a seed tray (38 × 24 cm) containing a layer of irradiated dune sand of 5 cm depth. *Plantago* seedlings were planted to form a central line through the trays and two outer rows of NM *Festuca* seedlings were placed on either side of the *Plantago* row. The distance between each seedling was 6 cm and the distance from the donor to the corner plants was 18 cm. The trays were placed in a growth chamber for 8 weeks, a period which previous studies had shown to be adequate to enable infection and spread to all M plants in a tray. At this time half of the seedlings in both M and NM trays were covered by a frame of aluminium foil for 48 hours to exclude light. During the shading period the shoot of the central *Plantago* plant in both the M and NM trays was sealed into a clear perspex box and 100 μCi of $^{14}CO_2$ was released around the shoots. After 48 hours of exposure to the gas the donor shoots were removed and all receiver shoots were harvested. Sand was carefully removed from roots and whole seedlings were air dried and prepared for autoradiography.

In both pot and tray experiments, quantitative analysis of distribution of isotope was carried out. Roots and shoots of the seedlings were processed in the same manner as ectomycorrhizal seedlings.

Results of analyses of the VA systems

A brief account of results obtained in the dish experiments has been provided elsewhere (Francis & Read 1984). Examination of dishes after removal of sand reveals that the VA mycorrhizal mycelium has grown from the donor root to form a network of hyphae covering the base of the dish in the M treatments. No differences could be detected between the patterns of mycelial development in sterilized and unsterilized sand. Hyphae making contact with receiver roots form VA infections provided that the contacts are made in regions of the root which are susceptible to infection. One of the most striking features revealed in these plant fungus associations is the distance across which hyphal connections are formed. Distances of several centimetres are commonplace (Fig. 5a, b). Some of the hyphae forming connections between roots have distinctive characteristics which have led us to call them 'arterial' hyphae. They have larger diameters, are less branched than other hyphae in the system and pass directly between roots (Fig. 5a, b). The situation is thus reminiscent of that seen in ectomycorrhizas where the formation of interconnections leads to enhanced development of the strands involved. In NM dishes virtually no mycelial development is observed, irrespective of whether the sand has been sterilized then exposed continuously to the open air, inoculated with saprophytes, or used in the field condition (Fig. 9a). This confirms the view that practically all of the mycelium observed in the mycorrhizal chambers is produced by the VA fungus.

Autoradiographic analysis of these dishes reveals that as in the case of ectomycorrhizal chambers, the tracer moves rapidly from donor plant into the mycelial network in the soil (Fig. 6a). Arterial hyphae become particularly heavily labelled (Fig. 7b). Radioactivity from these conduits accumulates first in the

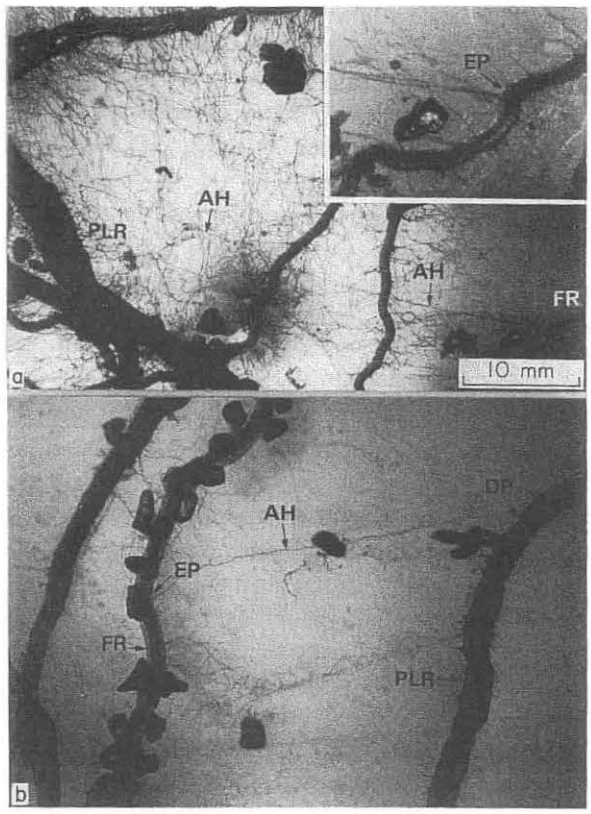

FIG. 5. Showing the development of vesicular-arbuscular mycorrhizal mycelium and the formation of inter-plant connections in interspecific associations of *Plantago lanceolata* and *Festuca ovina*. (a) Mycorrhizal mycelium has spread from previously infected *Plantago* 'donor' (PLR) plants to colonize the sand and infect *Festuca* 'receiver' roots (FR). An arterial hypha (AH) is shown passing over 4 cm from a donor root to infect a *Festuca* root (× 15). Inset: higher magnification of infection point on receiver root (× 30). (b) Arterial hypha (AH) passing from *Plantago* 'donor' root (PLR) to infect 'receiver' *Festuca* root (FR). Hyphal departure (DP) and entry points (EP) are marked. Adherence of sand grains to *Festuca* roots is caused by prolific root hair production in this species (× 38).

internal fungal mycelium and so provides clear demarcation of infected and uninfected areas of the root in the autoradiographs (Figs 6a and 8b). Other major sinks for assimilates are spores developing on the external mycelium (Figs 6a, b and 8b). Labelled material is later transferred from fungal structures and accumulates in root apices (Fig. 6b) as well as in the shoots of receiver plants (Table 3). The fungal vesicles within the root remain heavily labelled after the major transfer of label to host tissue has occured (Fig. 6b), the radioactivity presumably having accumulated in storage compounds.

Despite the very close proximity of roots of donor and receiver plants in the non-mycorrhizal systems (Fig. 9a), no transfer of radioactivity can be detected in autoradiographs of these associations (Fig. 9b). This demonstrates that even

TABLE 3. Radioactivity in shoots and roots of receiver plants (*Festuca ovina*) growing in pots containing *Plantago* as a donor, under three light regimes. Counts are expressed as d.p.m./mg dry weight (with 95% confidence limits) and as percentage of the total activity in the donor plants at harvest (n = 6). (From Francis & Read 1984)

Treatment	Receiver category: *Festuca:* Mycorrhizal			*Festuca:* Non-Mycorrhizal		
	Activity in root (d.p.m./mg dry wt)	Activity in shoot (d.p.m./mg dry wt)	Activity in whole receiver plant as % of that in donor	Activity in root (d.p.m./mg dry wt)	Activity in shoot (d.p.m./mg dry wt)	Activity in whole receiver plant as % of that in donor
Full light	8908** ± 3530	363*^{NS} ± 242	0·0151	656^{NS} ± 403	147^{NS} ± 194	0·0019
Half light	18072** ± 7647	479*^{NS} ± 75	0·048	264^{NS} ± 365	229^{NS} ± 372	0·0010
Dark	57218** ± 12372	51^{NS} ± 38	0·112	117^{NS} ± 100	ND*^{NS}	0·0005

* Indicates significant difference between this and all other values of activity at both treatment and category levels at $P<0·05$.

** Indicates significant differences between this and other figure so marked at $P<0·01$.

NS, Figure not significantly different from other treatments in the same category.

ND, No counts above background detected.

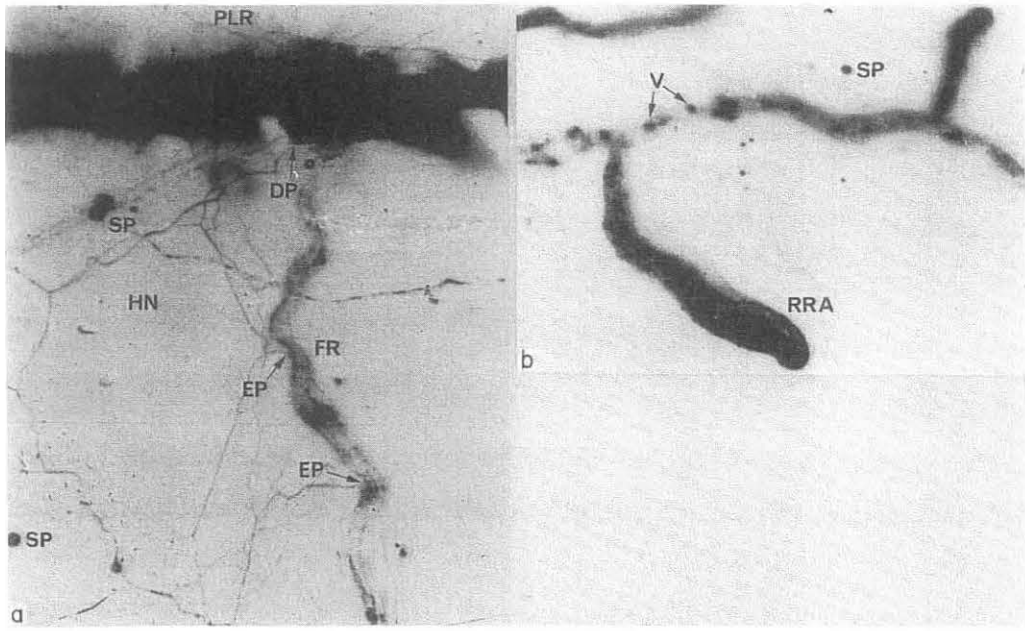

FIG. 6. Stripping film autoradiographs taken at different time intervals after feeding 'donor' plants of *Plantago lanceolata* with $^{14}CO_2$. (a) 48 hours after release of label over 'donor' shoots. Activity has moved from the heavily labelled donor root (PLR) into the hyphal network in sand (HN), and into infected regions of the *Festuca* 'receiver' root (FR). Departure (DP) and entry (EP) points are marked. Accumulation of label in spores is also seen (SP) (× 65). (b) At 72 hours after feeding of 'donor' plants, label has been transferred from infected regions of roots to receiver root apices (RRA). Activity remains high in external spores (SP) and internal vesicles (V) of the fungus (× 65).

though some leakage of carbon is to be expected from these roots, the magnitude of such loss bears little relation to the movement which is occurring into the mycorrhizal network. Facilitated uptake of leaked materials by scavenging mycorrhizal hyphae if it occurs at all, will therefore be a highly inefficient process, when compared to the direct transfer pathway revealed in the autoradiographs.

Quantitative determination of distribution of radioactivity confirms that label accumulates in roots of infected receiver plants and that it is eventually transferred to shoots (Table 3). The pattern of movement between mycorrhizal plants is greatly influenced by the light environment of the shoots, those kept in darkness during the isotope feeding period accumulating considerably more activity than those in half light, and half-light treatments in turn accumulating more than the full light.

Experiments with swards show that transfer between infected plants can occur at both intra- and interspecific levels over distances of at least 18 cm in 48 hours (Table 4a), and that in this circumstance also, shading has a major influence upon the pattern of assimilate distribution. The highest levels of activity are found in the roots of shaded mycorrhizal plants but a relatively small proportion of the activity is transferred to the shoot in this treatment. It may be that higher transpiration rates

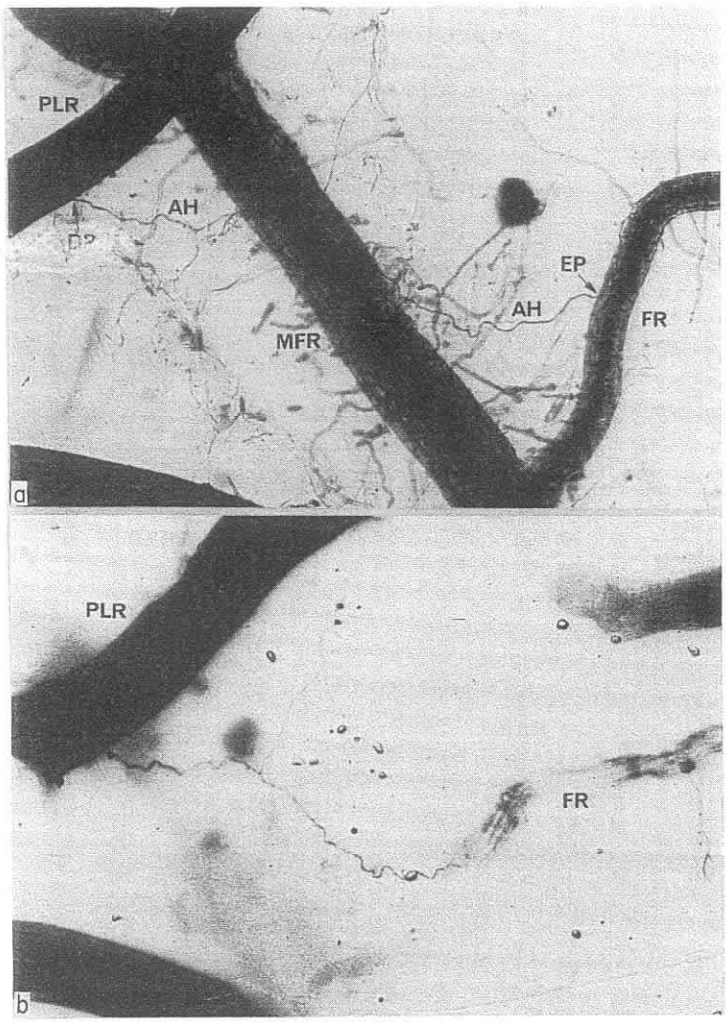

FIG. 7. Direct transfer of infection and of isotope through interconnecting hyphae in interspecific association of *Plantago* and *Festuca*. (a) Arterial hypha (AH) leaving donor root (PLR) at departure point (DP) and passing a mature *Festuca* root (MFR) which is not susceptible to infection and entering (EP) a young susceptible root (FR). Prolific root hair (RH) development is seen on *Festuca* roots (× 130). (b) Stripping film autoradiograph of (a) showing direct transfer of label form donor root (PLR) to infected area of receiver root (FR). (× 130).

in the more exposed unshaded part of the sward give rise to more rapid transfer from root to shoot. This possibility will be tested experimentally. Levels of activity in non-mycorrhizal trays are extremely low (Table 4b). Such results demonstrate that VA mycorrhizal mycelium provides a network in the soil through which all infected plants can exchange carbon, the direction of greatest net movement being determined by shade and hence physiological need.

TABLE 4(a). Distribution of plants and of radioactivity (d.p.m. mg d wt^{-1}) in simulated sward of mycorrhizal *Festuca* (square symbols) and *Plantago* (circular symbols) plants, 48 hours after feeding the central *Plantago* donor (PL. DONOR) with $^{14}CO_2$. Closed symbols represent shaded plants, open symbols fully illuminated plants. The upper figure at each plant is shoot radioactivity, the lower root radioactivity. (b) Details as for (a) but in sward of non-mycorrhizal plants.

(a) *Mycorrhizal sward*

Shoot	616	122	9	556	656
	■	■	●	□	□
Root	736	268	374	215	612
	625	366	42	565	743
	■	■	●	□	□
	15681	13750	2442	2142	1134
	412	135	PL. DONOR	426	639
	■	■	★	□	□
	45072	87555		7233	3162
	260	435	109	303	181
	■	■	○	□	□
	88997	102070	1183	7212	1659
	118	56	47	230	133
	■	■	○	□	□
	5580	4490	1370	2238	2405

(b) *Non-mycorrhizal sward*

Shoot	21	235	15	30	70
	□	□	●	■	■
Root	15	0	46	143	300
	13	5	0	3	65
	□	□	●	■	■
	19	36	7	118	50
	0	7	PL. DONOR	12	27
	□	□	★	■	■
	0	20		19	21
	32	1	49	318	12
	□	□	○	■	■
	23	42	220	81	112
	13	40	10	12	425
	□	□	○	■	■
	47	57	5	3	0

DISCUSSION

Though large numbers of studies of the structure and function of mycorrhizal roots have been carried out (see recent review by Harley & Smith 1983) relatively little attention has been given to the mycelial phase of the symbiosis in the soil. The main reason is probably that while entire mycorrhizal roots or even root systems can readily be extracted from soil with little damage, it is extremely difficult to carry out a non-destructive investigation of the mycelial system. Nevertheless, the importance of the external mycelia for the nutrition of individual plants with both ecto- and VA mycorrhizas has been demonstrated.

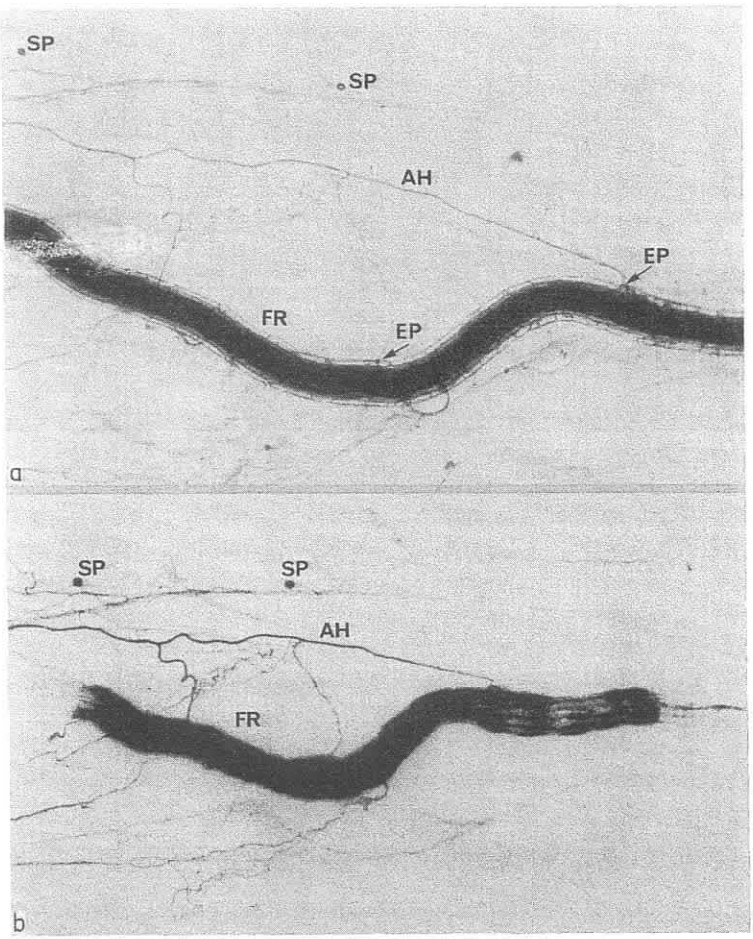

FIG. 8. Distal portion of arterial hyphae showing points of entry (EP) into receiver roots (FR). (a) Arterial hypha (AH) of relatively larger diameter branching in the vicinity of a receiver root (FR) to form two entry points (EP) (× 130). (b) Stripping film autoradiograph of this region showing heavy labelling of the arterial hypha (AH) and of spores (SP) in the external mycelium, and of the zone of infection within the receiver root (FR) (× 130).

Melin & Nilsson (1950, 1952, 1953) clearly showed that ectomycorrhizal mycelia growing in pure culture with seedlings could absorb nutrients and provide channels for their transport to roots. The occurrence of mycorrhizal strands in soil, at considerable distances from the roots to which they were attached, was later demonstrated by Schramm (1966). Bowen (1973) pointed out that such strands should enhance plant nutrient uptake largely by growing though the depletion zones which surround roots, into nutrient-rich regions beyond the rooting area. It was later shown (Skinner & Bowen 1974) that mycelial strands attached to roots of *Pinus radiata* are able to absorb phosphate ions which were then translocated to mycorrhizal roots.

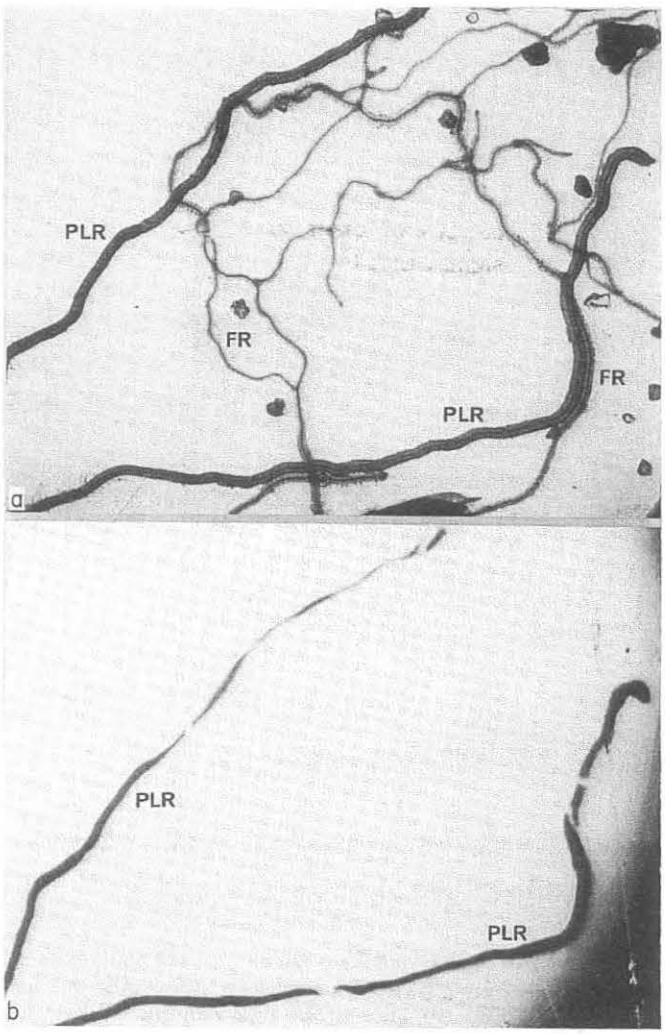

FIG. 9. Light micrograph and autoradiograph of non-mycorrhizal root chamber. (a) Closely intermingled roots of *Plantago* (PLR) and *Festuca* (FR) are shown. Very few hyphae are seen in such systems even when the plants are grown under unsterile conditions (× 13). (b) Stripping film autoradiograph of this system taken after feeding *Plantago* shoots with $^{14}CO_2$ under the same circumstances as those used for M systems. No transfer of radioactivity from heavily labelled *Plantago* roots (PLR) is detectable even though extensive physical contact between donor (PLR) and receiver (FR) roots is seen in (a) (× 13).

The major function of the ectomycorrhizal sheath appears to be that of nutrient storage (Harley & McCready 1950, 1981; Bowen & Theodorou 1967). Nutrients such as phosphate are released to the soil solution in seasonal flushes of microbial activity, P ions being captured and stored in the fungal sheath in the form of polyphosphate granules (Harley & McCready 1981). Since the capacity of mycorrhizal roots to absorb phosphate is as much as five times greater than that of

their non-mycorrhizal counterparts (Harley & McCready 1950), the sheath must play a major role in the storage and conservation of such nutrients. The quantity of mycelium external to the sheath differs in different host species. Thus, while the external mycelial system of pine would be expected to play a major role in the capture of phosphate and its transport to the sheath, such a system is relatively poorly developed in beech forest (Harley 1978) where the sheath has few attachments to the soil. This difference in pattern of distribution of external mycelium, which is likely to be of considerable significance for the soil ecosystem as a whole (Harley 1978; Read 1984), may arise as a result of the different patterns of nutrient release in coniferous and deciduous tree litter. Nykvist (1963) showed that assimilates in fresh leaf litter of several deciduous tree species could be readily leached by water, but that losses from coniferous litter were slower and occurred over a longer time span. Whereas an extensive external network of mycelium might provide an energetically efficient absorptive system in the coniferous situation, the maintenance costs might be prohibitive in the case of the ephemeral nutrient release pattern asociated with the deciduous litter.

Analysis of the development of mycelial systems in root chambers shows that as well as being nutrient-absorbing structures the strands are responsible for the initiation and spread of infection. Their role in this capacity in the field has recently been demonstrated by Fleming (1983) who showed that whereas in an agricultural soil a number of non-strand-forming fungi may act as mycorrhizal colonists of seedling birch roots, in the forest the main mycorrhiza formers are strand-forming fungi which are almost certainly growing from roots of the older forest trees. There is thus indirect evidence that seedlings in the forest are quickly integrated into the common mycelial system.

The importance of ectomycorrhizal fungi in the soil ecosystem was stressed by Harley (1978) who suggested that their mycelia might be the source of the considerable quantities of CO_2 evolved in soil 'respiration' which could not be accounted for in terms of decomposition processes. Recalculating data provided by Romell (1939), Harley showed that mycorrhizal fruit body production alone could utilize carbon equivalent to as much as 10% of that required for annual timber production. Studies at the ecosystem level (Fogel & Hunt 1979; Vogt *et al.* 1982) have confirmed Harley's view. In Douglas fir forest, Fogel & Hunt found that up to 50% of the annual throughput of dry matter takes place in the fungal component of the forest and that 23% of this takes place through the hyphae, most of which can be assumed to be associated with mycorrhizas. Vogt *et al.* (1982), working in *Abies amabilis* forests, showed that fungal reproductive structures could account for up to 15% of net primary production of the stands. Since the fruit bodies of most mycorrhizal fungi are relatively ephemeral structures it seems likely that in most cases the vegetative mycelium will contribute an even greater sink for carbon over the full growing season. The analysis of structure and function of the external mycelium of ectomycorrhizal roots helps to explain the massive investment of carbon in this part of the system. It appears that in many members of the Pinaceae, at least, the major function of absorption from soil is fulfilled by the external

mycelium which acts as a physiological extension of the root system. The function of the root thus becomes largely one of anchorage and this in turn may explain the exceptionally low root densities observed in pine stands by Kramer & Bullock (1966), who found only 5–50 cm of root length per cm^2 of ground surface.

While the spreading mycelial fans which grow into soil around young mycorrhizal roots clearly provide intensive soil exploitation, the strands which differentiate within them are better equipped to act as channels for transport of nutrients. Thus, while the early stages of mycelial growth will optimize nutrient capture, the mature phase will provide for efficiency of resource distribution and conservation. Reid & Woods (1969) demonstrated that interplant transfer of carbon occurred when a cut mycelial strand was wrapped around a 'receiver' root. Studies in our laboratory (Duddridge *et al.* & 1980; Brownlee *et al.* 1983; Read 1984) now indicate that the mycelial strands form an interconnecting system of direct pathways through which water and carbon flow using channels which are functionally analogous to xylem and phloem.

In VA as in ectomycorrhizal plants it is widely accepted that the enhancement of plant growth which arises from infection is attributable to the improved soil exploitation provided by the external mycorrhizal mycelium. Increased rates of phosphate uptake and inflow to plant roots have generally been considered to be the major consequence of VA mycorrhizal infection (Sanders & Tinker 1973; Pearson & Tinker 1975; Sanders *et al.* 1977) though it has recently been shown that VA mycelium can also significantly enhance absorption of the ammonium ion (Ames *et al.* 1983).

Despite early appeals for more information on the ecology of the external phase of the VA symbiosis (Harley 1950), little progress has been made to date and evidence for a positive relationship between the quantity of external mycelium and levels of growth enhancement has only recently been provided (Sanders *et al.* 1977; Graham, Linderman & Menge 1982; Bethlenfalvay, Brown & Pacovsky 1982). In all these studies the methods of determining mycelial quantities are destructive so the pattern of distribution of hyphae in soils remains uncertain. Using washing methods Nicolson (1959) and Mosse (1959) extracted mycelium from soil and provided useful descriptions of the types of hyphae present. Both workers were impressed by the quantities of mycelium extracted and they observed a tendency for dimorphism in the hyphae. Thick-walled coarse hyphae with angular projections and diameters up to 20 μm were the main structural elements and these give rise to lateral branches with thin walls and narrow diameters (2–7 μm). The relatively smooth and unbranched 'arterial' hyphae which form the major interconnections between plants in the root chambers were not described by these workers, probably because they would be broken and deformed on extraction. It may be that the arterial hyphae become thick-walled and coarse with age but it is of interest that even the thick-walled structures observed by Nicolson had cytoplasmic contents indicating that they were alive and probably attached to a living root. Analysis of $^{14}CO_2$ distribution through the mycelium also indicates that the bulk of the mycelium is alive, the extra-matrical vesicles in particular being major sinks for

labelled assimilate. Cytoplasmic continuity would be a prerequisite for distribution of assimilate through the mycelium. If, as seems likely, the bulk of the external mycelium of VA mycorrhizal roots is viable it must constitute a nutrient resource of great significance for the soil biota, some components of which graze extensively on mycorrhizal mycelium (Warnock, Fitter & Usher 1982). Measurements of hyphal length of VA fungi associated with soil aggregates in loam (Tisdall & Oades 1979) have revealed up to 55 m of hyphae per gram of soil. Thus, apart from being a major food resource in soil the mycelium may also be largely responsible for the stabilization of the soil aggregates.

The study of VA mycelium in root chambers shows that it has a range of functions comparable with those seen in ectomycorrhizal systems. The hyphae are largely responsible for the spread of infection within and between plant root systems and also provide channels for interplant transfer of nutrients. Heap & Newman (1980) showed that when ^{32}P leaked from dying roots it was more rapidly taken up by nearby plants if they were mycorrhizal. While this type of enhanced nutrient absorption could arise as a result of hyphal scavenging in the vicinity of the dying roots and thus be a form of facilitated uptake, evidence of direct transfer of materials between living plants—which is analogous to that seen in the ecto-mycorrhizal associations—is now available (Francis & Read 1984; see also Fig. 4). Such a transfer process must be more efficient than that involving facilitated uptake because the nutrients are absorbed from the intracellular pools, to which the mycorrhizal fungus alone has access, and are transported to distant sinks without being released at any stage to the saprotrophic microflora of the soil. It is likely that the direct transfer patterns will predominate in situations where living mycorrhizal plants are connected and that the direction and magnitude of transfer of materials such as carbon will be determined by gradients of sink size.

Transfer of ^{32}P between living mycorrhizal plants has recently been demonstrated in the field (Chiariello, Hickman & Mooney 1982), and it has been shown in pot experiments (Whittingham & Read 1982) that the flux of materials from nutrient-enriched mycorrhizal source plants to starved receiver seedlings was sufficient to induce significant growth responses in these seedlings.

A new picture of the mycorrhizal mycelium in the soil thus emerges. Interconnections arising at the time of infection of a young root are maintained in a functional condition after their formation. In both ecto- and VA mycorrhizas, carbon has been shown to move freely through the interconnections along gradients of concentration, and it seems likely that both mineral nutrients and water can move by way of the same direct mycelial pathway between plants at both the intra- and interspecific levels.

The observation chambers show that a very large mycelial biomass can be sustained by such transfer processes under both ecto- and VA host plants. Modern methods of determination of soil microbial biomass (Anderson & Domsch 1973) probably lead to a great underestimation of the importance of this quite massive component of the soil microflora because the mycorrhizal mycelia are fragmented, and detached from all natural carbon sources in the course of the assay. Access to

direct supplies of carbon would be expected to provide significant competitive advantages to mycorrhizal biotrophs growing through soil. Gadgil & Gadgil (1975) provided indirect evidence for the presence of such an advantage by showing that the activities of litter decomposing saprotrophs was inhibited in the presence of mycorrhizal roots. We are currently undertaking a comparative analysis of respiratory activities in intact mycorrhizal and non-mycorrhizal root chambers with a view to providing discrimination between the activities of the biotrophic and saprotrophic components of the soil microflora.

The occurrence of direct interplant transfer of carbon is likely to be of considerable significance to the physiology of the interconnected plants. It is known that seedlings of some species of both woodland and grassland habitats can survive prolonged periods of exposure to deep shade (Salisbury 1930; Chippindale, 1932). When growing in nutrient-poor soil some of these plants are even able to survive for long periods in complete darkness (Hutchinson 1967). Most of the species shown to be strongly shade-tolerant in such studies would be expected to be mycorrhizal in the field and it is evident that their survival in nature could be assisted by transfer of resources from illuminated over-storey plants. Mahmoud & Grime (1974) proposed that the remarkable shade tolerance of the grass *Deschampsia flexuosa* is attributable to its inherently low respiration rates. Clearly, again, the provision of additional respiratory substrates from nearby plants through mycorrhizal mycelia could contribute to the survival of plants in stressed circumstances of this kind. The experimental results described above suggest that carbon will move readily from well illuminated over-storey plants to shaded seedlings. The chance that seedlings will receive access to this supply even in a community of mixed species is enhanced by the lack of mycorrhizal specificity. Even if the amount of assimilate received were sufficient only to sustain the mycorrhizal association the elimination of such a respiratory drain would be a significant advantage for a shaded seedling. Autoradiographic and quantitive analyses reveal, however, that labelled assimilate is moved from regions of infection to root apices and to the shoots of infected plants so the benefits are distributed more widely.

Internal recycling of nutrients is a well known mechanism for the redistribution and conservation of resources within individual plants, but the possibility that resources may pass between individuals within the community has not been widely recognized. Resource transfer of this kind arises as a secondary but nonetheless important consequence of infection of the individual plants. Selection for the mycorrhizal habit in individuals probably arose because of the improvement of nutrient status and survival potential which infection provided. The formation of connections between individuals and species—with all of the repercussions in relation to patterns of nutrient circulation within the plant community—inevitably follows, both because of the low levels of host specificity shown by most mycorrhizal fungi, and because the normal method of infection involves growth of a hypha from a resource base on one root to a susceptible uninfected root elsewhere in the soil. Restriction of specificity in the fungus would be favoured by natural

selection since it increases the chance that the heterotroph will obtain access to a suitable nutrient source.

There is normally an inverse relationship between the intensity of mycorrhizal infection and the fertility of the soil. As a result, the numbers of mycelial interconnections and, therefore, the potential for interplant transfer of nutrients will be greatest in those infertile situations where the benefits of such transfer would be most strongly felt. Many unproductive soils have high species densities. Grime (1973) has suggested that the ability of species to coexist in these environments is derived from the capacity of individuals to persist for long periods of time despite the low nutrient status of the soil environment, rather than from their competitive abilities. Transfer of nutrients from established plants to seedlings may be a crucial factor enabling young plants of a range of species to survive in these circumstances.

Direct nutrient transfer pathways have the further significant advantage that they greatly improve nutrient conservation at the ecosystem level. The current nutrient capital of the system is retained in circulation between the autotrophs, thus restricting losses which inevitably arise through leaching or microbial immobilization when such resources enter the soil system. The evidence obtained so far confirms the existence of the direct transfer pathway in both ecto- and VA mycorrhizal systems. Further studies are now required to determine the quantitative significance of the transfer process under a range of environmental circumstances.

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